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TITLE: Magnetic Resonance Spectroscopy (MRS) of Prostatic Fluids for Early Detection of Prostate Cancer

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14. ABSTRACT

The prostate gland, its tissues and fluids, have unique metabolic profiles due to specific physiological functions. In this study, proton nuclear magnetic resonance spectroscopy (1H-NMRS) is used to analyze potential metabolic markers of prostate cancer (PCa) in human expressed prostatic secretions (EPS). To date, metabolic profiles of EPS from 52 men with PCa and from 26 healthy controls have been analyzed. The metabolites analyzed included citrate, spermine, myo-inositol, lactate, alanine, phosphocholine, glutamine, acetate, and hydroxybutyrate. Absolute concentrations have been quantified using a novel method developed by our Co-Investigator. The results to date indicate that citrate, myo-inositol and spermine are potentially important markers of PCa. Further, the absolute concentrations of these metabolites in EPS appear to be independent of age, increasing the potential utility of these markers due to elimination of age as a confounding variable. Ongoing activities include the prospective validation of these promising results and analysis to determine if these metabolic markers can distinguish between aggressive tumors and those less aggressive.

15. SUBJECT TERMS
prostate cancer, magnetic resonance spectroscopy, expressed prostatic secretions, early detection, screening, predictive modeling

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INTRODUCTION:

Due to specific physiological functions, prostatic tissues and fluids have unique metabolic profiles. In this study, proton nuclear magnetic resonance spectroscopy (^{1}H-NMRS) is used to analyze potential metabolic markers of prostate cancer (PCa) in human expressed prostatic secretions (EPS). The scope of this research includes utilizing advanced statistical methods to identify metabolic profiles in EPS that are consistent with the presence of prostate cancer. EPS samples from men with PCa are being compared with EPS from healthy controls. The metabolites analyzed included citrate, spermine, myo-inositol, lactate, alanine, phosphocholine, glutamine, acetate, and hydroxybutyrate. Absolute concentrations are quantified using a novel method developed by our Co-Investigator. In addition to identifying markers of PCa, we seek to identify markers that will distinguish between high-grade (aggressive) tumors and low-grade (less aggressive) tumors. Ongoing activities include the prospective validation of promising preliminary results. It is hoped that the metabolic profiles identified through this research will improve the sensitivity and specificity of prostate cancer screening thereby benefiting men at risk for prostate cancer by increasing the number of cancers detected early while reducing the number of false-positive tests.

BODY:

Methods:

Patient Population and Sample Collection. The 78 EPS samples analyzed in this study were obtained from the biorepositories of two institutions and were collected under IRB approved protocols. All of the EPS donors signed informed consent forms agreeing to donate their EPS and agreeing that the samples would be used in prostate cancer research. All samples were obtained via transrectal prostate massage and were spun down at 13,000 g at 4°C for 5 minutes to remove cell debris immediately upon collection. The supernatant was stored at -80 °C until needed for analysis. Of the 78 samples analyzed, 52 were from men with prostate cancer and 26 were from healthy (non-PCa) controls.

Sample Preparation and Quantitative ^{1}H-NMRS analysis. Frozen samples were slowly thawed at 4 °C. Depending on the initial volume, 5 to 20 µL of EPS were analyzed. Deuterium oxide (D_2O, 20 to 35 µL), containing trimethylsilyl propionic-2,2,3,3,-d4 acid (TMSP) as an external standard, was added to each EPS sample resulting in final sample volume of 40 µL. The samples were centrifuged at 4,000 g at 4 °C for 5 min. The supernatants were transferred into Bruker 1-mm glass capillaries (Bruker Biospin, Fremont, CA) using 1-mL syringes with thin epidural needles. The glass capillaries were sealed and inserted into the magnet using a 1-mm NMR spinner.

All ^{1}H-NMRS analyses were performed by NMR scientists (NJS, DJK) who were "blinded" to the clinical background of the EPS samples. All EPS samples were analyzed using a Bruker DRX 500 MHz high-resolution NMR spectrometer (Bruker Biospin, Fremont, CA). The sample temperature was held constant at 5 °C inside of the magnet using a Bruker temperature regulator. Bruker 1-mm TXI micro-probe for ultra-small volume samples was used for all experiments. Deuterium lock signal was held for D_2O added to the sample. All ^{1}H-NMR spectra were obtained using XWIN-NMR 3.5 or TopSpin software (Bruker Biospin, Fremont, CA). For metabolite quantification, a standard proton water pre-saturation pulse program "zgpr" was used to suppress water residue signal. The resonance frequency for proton channel was 500 MHz with 80 total acquisitions. A pulse delay of 12.8 seconds was applied between acquisitions for fully relaxed ^{1}H-NMR spectra (calculated as 5*T1). The total acquisition time was 20 minutes, 45 seconds. TMSP was used as an external reference for metabolite quantification and chemical shift (0 ppm). The absolute concentration of TMSP in deuterium oxide was verified for each experiment set using 20 mM amino acid solution (citrate, alanine, myo-inositol, glutamine) as a quantification standard. Endogenous EPS metabolites were identified from 2D-NMR spectra (COSY, HSQC) based on the results from our chemical-shift database. After performing Fourier transformation (with line broadening LB=0.1 Hz) and making phase and baseline corrections, each identified ^{1}H peak was integrated using the Bruker 1D WIN-NMR version 4.0 program. The absolute concentrations of single metabolites were then referred to the TMSP integral and calculated according to the equation:

$$Cx = \frac{Ix : Nx \times C}{I : 9} \times V : Veps \quad (1)$$

where Cx = metabolite concentration
Ix = integral of metabolite ^{1}H peak
Nx = number of protons in metabolite ^{1}H peak (from CH, CH_2, CH_3, etc.)
C = TMSP concentration calculated for each experiment set
I = integral of TMSP ^{1}H peak at 0 ppm (:9 since TMSP has 9 protons)
V = total volume of the sample (40 µL)
$Veps$ = volume of EPS sample (5 to 20 µL)

Since in ^{1}H-NMR spectroscopy, single metabolites can produce multiple peaks, the final concentration for each metabolite was calculated as an average of its ^{1}H-NMR peaks. The absolute concentrations of metabolites were reported as µmol per mL of EPS.

Metabolite Stability Analysis. To assess any possible metabolic degradation occurring during freezing, -80 °C storage, or thawing procedures, we analyzed 6 freshly collected EPS samples at three different time points post-collection. Each of the 6 fresh EPS samples was divided into three aliquots (10 µL each). The first aliquot was analyzed by ^{1}H-NMRS immediately after sample collection (day 0), the second aliquot was analyzed after 1 week of -80 °C storage (week 1), and the third after 1 month of -80 °C storage.

Statistical Analysis. The statistical modeling and analysis group (RHJ, EJG, CO) took no part in the MRS analysis and were the only staff members who had access to the clinical and outcome data.

The primary statistical method utilized was logistic regression using SAS (2004) PROC GENMOD. The analysis was carried out separately for each metabolite with the binary response being cancer or no cancer. The log of the metabolite concentration was the independent variable, and age was included in the model to control for the age difference between the group with cancer and the group without cancer

SAS PROC MIXED was used to analyze the differences between metabolite concentrations due to freezing and thawing in the metabolite stability analysis. The response variable for each metabolite was the log of the concentration of the metabolite. The independent variables were an intercept and a 0, 1 indicator variable for time 1 and a 0, 1 indicator variable for time 2. A random subject effect was included in the model since there are repeated measures on each subject, and this allows each subject to have a different mean level. The hypothesis to be tested is whether the two coefficients of the indicator variable are zero, which would indicate that there is no effect from freezing the samples.

RESULTS

The characteristics of the men who provided EPS samples are summarized in Table 1. Biopsy Gleason sums and serum PSA levels were only available for the men with cancer. The average age of the EPS donors with PCa was 58.0±7.0 years, and was 52.2±12.1 for the healthy donors. The difference in mean ages between the men with cancer and the healthy controls was just statistically significant (p = 0.04).

Table 1: EPS donor characteristics.

	All Subjects	With Cancer	Controls
N	78	52	26
Age in years – mean (SD)	56.1 (±9.4)	58.0 (± 7.0)	52.2 (± 12.1)
	Median (range)	Median (range)	Median (range)
Biopsy Gleason Sum	na	7 (5 – 9)	na
PSA in ng/ml	na	5.4 (2.0 – 28.0)	na
[Citrate] in µmol/ml	240.1 (14.3 – 764.5)	137.2 (14.3 – 444.4)	353.2 (125.9 – 764.5)
[Spermine] in µmol/ml	43.8 (2.1 – 168.2)	30.6 (2.1 – 133.3)	58.0 (18.9 – 168.2)
[Myo-Inositol] in µmol/ml	13.6 (.81 – 41.9)	7.2 (.81 – 26.6)	21.2 (7.7 – 41.9)
[Lactate] in µmol/ml	1.1 (.00* - 13.2)	1.0 (.14 – 13.2)	1.1 (.00* - 7.9)
[Alanine] in µmol/ml	.47 (.00* – 5.8)	.38 (.01 – 5.4)	.70 (.00* - 5.8)
[Posphocholine] in µmol/ml	.28 (.01 – 4.6)	.07 (.01 – 4.2)	.42 (.02 – 4.6)
[Glutamine] in µmol/ml	1.1 (.00* - 9.6)	1.1 (.01 – 9.3)	2.0 (.00* - 9.6)
[Acetate] in µmol/ml	.00* (.00* – 2.9)	.09 (.00* – 2.9)	.00* (.00* - 00*)
[Valine, Leucine] in µmol/ml	13.1 (.86 – 37.9)	9.9 (.86 – 37.9)	16.6 (7.7 – 37.7)
[Hydroxybuterate] µmol/ml	.60 (.00* - 7.9)	.40 (.01 – 5.4)	.78 (.00* - 7.9)

EPS = expressed prostatic secretions; N = number of subjects; SD = standard deviation; PSA = serum prostate specific antigen; [x] = absolute concentration of x; na = not applicable; *= not detectable

Table 2 provides a summary of the LR modeling results and includes p-values for coefficients of log of the metabolite concentrations. A small p-value indicates that the log of the metabolite concentrations are predictive of PCa. The p-values for age indicate the effects of controlling for age. A large p-value for age such as the ones for citrate, myo-inositol and spermine indicate that controlling for age for these variables is not necessary. The logistic regressions for these three variables are then refitted without age in the model. The deviance over the degrees of freedom, also included in Table 2, provides a measure of how well the given LR model was fitted. Values near 1 indicate that the models fit well. A value of the deviance over the degrees of freedom greater than one suggests under-fitting. Based upon the results of the LR modeling, the log of the absolute concentrations of citrate, myo-inositol, and spermine were the most predictive of prostate cancer, while not being dependant on age. At 90% sensitivity, these metabolites had specificities of 74%, 51% and 34%, respectively. The areas under the receiver operating characteristic curves (AUROC) for citrate, myo-inositol and spermine were 0.89, 0.87, and 0.79, respectively. Figure 1 provides the ROC curves for these three metabolites.

Table 3 summarizes the results of the metabolite stability analysis on six samples. Two ^{1}H-NMR peaks each for citrate and spermine were measured. The concentration of Alanine was below the ^{1}H-NMRS lower limit of quantification in one of the EPS samples. Therefore, five measurements of alanine from the six samples analyzed were available for comparison. The p-values greater than 0.05 indicate that no statistically significant changes in metabolite concentrations occurred during the various freeze-thaw cycles for a given metabolite. Thus, only glutamine and alanine underwent significant changes in concentrations (degradation) between freeze-thaw cycles. No metabolic degradation was observed for hydroxybutyrate, citrate, myo-inositol, lactate, phosphocholine, spermine, or valine in fresh versus frozen samples

Table 2. Logistic regression analysis of EPS metabolites in relation to prostate cancer and age.

Log Base 10 of:	p-value	deviance/DF	p-value (age)
Citrate	0.0005	0.7762	0.9598
Myo-inositol	0.0006	0.8295	0.7368
Spermine	0.0038	1.0811	0.2582
Valine - Leucine	0.0043	1.0983	0.079
Citrate/Lactate	0.0013	0.9977	0.0348
Citrate/Spermine	0.0003	0.9912	0.0325
Posphocholine	0.0475	1.2373	0.0236
Citrate/phosphocholine	0.9586	1.2775	0.0152
Citrate/inositol	0.0823	1.2266	0.0131
Alanine	0.0659	1.2075	0.0102
OH-Butyrate	0.0125	1.1479	0.0093
Glutamine	0.0258	1.1176	0.0087
Lactate	0.5308	1.262	0.0082

EPS = expressed prostatic secretions; PCa = prostate cancer; DF = degrees of freedom

Table 3. Metabolite stability analysis results (6 samples).

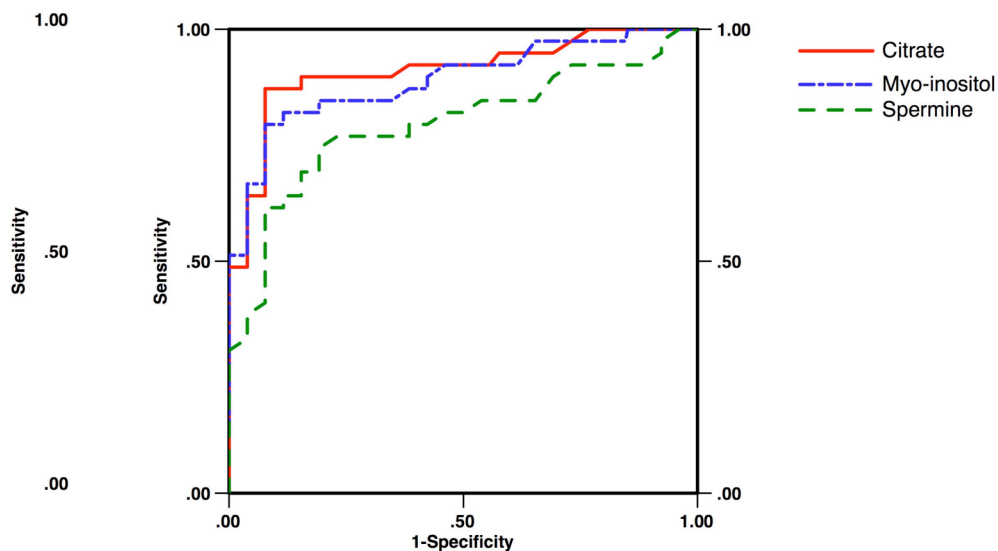| Metabolite	Chemical Shift	Overall p-value
Myo-Inositol	4.07 ppm	0.2418
Phosphocholine	3.24 ppm	0.2157
Spermine1	3.20 ppm	0.4280
Citrate1	2.72 ppm	0.2763
Citrate2	2.58 ppm	0.4427
Glutamine	2.36 ppm	**0.0413**
Spermine2	2.11 ppm	0.9197
Spermine3	1.78 ppm	0.0719
Alanine	1.45 ppm	**0.0261**
Lactate	1.32 ppm	0.4254
OH-Butyrate	1.19 ppm	0.1165
Valine - Leucine	1.01 ppm	0.6667

ppm = parts per million

Figure 1. Receiver operating characteristic (ROC) curves for citrate, myo-inositol, and spermine.

In addition to the results for the absolute concentrations of metabolites provided above, the ratios of the metabolite concentrations (relative concentrations) were also analyzed. Using relative concentrations (metabolite ratios) in a two-variable LR model, citrate/spermine and citrate/lactate were also predictive of prostate cancer with an area under the receiver operating characteristics curve of 0.76 (95% CI 0.71-0.81).

KEY RESEARCH ACCOMPLISHMENTS:

- Confirmed reports in the literature of the value of the relative concentration of Citrate in EPS as a potential marker of prostate cancer.

- Confirmed reports in the literature of the value of the relative concentration of Spermine in EPS as a potential marker of prostate cancer.

- Found that – in a two-variable LR model - citrate/spermine and citrate/lactate were also predictive of prostate cancer, but to a lesser degree than the absolute concentrations of these metabolites.

- Contradicted reports of other researches, which have indicated that phosphocholine concentration is a marker of cancer. We found no correlation between phosphocholine concentrations in EPS and the risk of prostate cancer.

- Validated the use of frozen EPS via a sub-study where we found no significant metabolic degradation for hydroxybutyrate, citrate, myo-inositol, lactate, phosphocholine, spermine, or valine in samples that were frozen and thawed at 1 week and at 1 month as compared to freshly collected EPS. Only the metabolites alanine and glutamine showed significant changes in concentrations, but were not metabolites of interest in this study.

- Utilized a novel method developed by Co-Investigator N. Serkova to measure the absolute concentrations of metabolites (previous studies have only measured relative concentrations of metabolites in EPS).

- Established a quantitative and validated 1H-NMRS protocol to analyze ultra-small volume human body fluids using a 1-mm micro-probe.

- Found that absolute concentrations of citrate, myo-inositol, and spermine are predictive of prostate cancer while being independent of age.

EPS = expressed prostatic secretions

LR = logistic regression

REPORTABLE OUTCOMES:

Peer-Reviewed Abstracts, Presentations, and Manuscripts:

Nuclear magnetic resonance spectroscopy of expressed prostatic secretions: Metabolite citrate and derivatives are potential markers of prostate cancer.

Natalie Serkova (presenter), Eduard J Gamito, Richard H Jones, Colin O'Donnell, Tammy Hedlund, E. David Crawford.

Abstract <u>presented</u> (Abstract and Oral Presentation) at the 16th International Prostate Cancer Symposium, Beaver Creek, Colorado, January 2006

Validation of citrate and derivatives in expressed prostatic secretions to predict prostate cancer: High-resolution ^{1}H-NMR study.

Natalie J. Serkova (presenter), Eduard J. Gamito, Richard H. Jones, Colin O'Donnell, E. David Crawford.

Abstract <u>presented</u> (poster session) at the 97th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 2006.

High-resolution nuclear magnetic resonance spectroscopy of expressed human prostatic secretions: The metabolites citrate, myo-inositol, and spermine are potential age-independent markers of prostate cancer.

Eduard J Gamito (presenter), Natalie Serkova, Richard H Jones, Colin O'Donnell, Tammy Hedlund, E. David Crawford.

Abstract <u>accepted</u> for Discussed Poster session at the Annual American Urological Association meeting, Atlanta, Georgia, May 2006.

Nuclear magnetic resonance spectroscopy of expressed prostatic secretions: Metabolite citrate and derivatives are potential markers of prostate cancer: An Update.

E. David Crawford (presenter), Natalie Serkova ,Eduard J Gamito, Richard H Jones, Colin O'Donnell, Tammy Hedlund,.

Abstract <u>accepted</u> for Poster Session at the Annual American Society of Clinical Oncologists, Atlanta, Georgia, June 2006

High-resolution nuclear magnetic resonance spectroscopy of expressed human prostatic secretions: The metabolites citrate, myo-inositol, and spermine are potential age-independent markers of prostate cancer.

Natalie J. Serkova, Eduard J. Gamito, Richard H. Jones, Colin O'Donnell, Douglas J. Kominsky, Jaimi L. Brown, Spencer Green, Holly Sullivan, Tammy Hedlund, E. David Crawford.

Full-length manuscript <u>in progress</u> to be submitted to the Journal of Clinical Oncology in May, 2006.

CONCLUSION:

Important Findings:

Perhaps the most important and novel finding of this study is that the absolute concentrations of citrate, myo-inositol and spermine are predictive of prostate cancer while being independent of age. While previous studies have indicated that ratios of citrate to other metabolites (relative concentrations) are potentially predictive of prostate cancer, the current study, to the best of our knowledge, is the first to indicate that these three metabolites are independent of age. This is important because age is a strong confounder in the association between prostate specific antigen (PSA) – the most widely used marker of prostate cancer - and prostate cancer risk. Moreover, benign prostatic hyperplasia (BPH), which is also strongly associated with age, can raise PSA levels and further muddy the waters in terms of the early detection of prostate cancer. Thus, age-independent markers of PCa have the potential to improve early detection and diagnostics by eliminating this important confounder while reducing false-positives and their associated harms.

Potential Limitations:

While these results are promising, caution is warranted. The primary limitation of the current study is the small number of samples analyzed (65) in relation to the number of metabolites measured (9). Ratios of samples to variables such as these have the potential to lead to false associations being made between variables due to chance. Another potential limitation of this study is the possibility that the logistic regression (LR) models over-fit the data used to develop the models. Over-fitting is a phenomenon where a model describes the available data well, but is not able to generalize effectively with new data. The deviance/DF measures provided in Table 2 suggest that over-fitting may have occurred in the LR modeling of citrate and myo-inositol, but not in spermine. A prospective validation of these models, which is underway, will be necessary to confirm the current results.

Additional work to be done under the current grant:

As mentioned, the promising results outlined above must be prospectively validated. As part of our efforts to complete the current project, we are requesting additional EPS samples from our biorepository to prospectively validate our LR models. To accomplish this, our MRS team will analyze and produce MRS spectra for the new samples. Whether the samples come from men with or without prostate cancer will be blinded to both the MRS group and the statistical group. The performance of the LR models will be "graded" to determine if, indeed, they are able to generalize to new data. If our preliminary results hold up under prospective validation, we will seek additional funding to complete a larger, multi-institutional study to further confirm these results. We believe the interest in an age-independent marker of prostate cancer would be high in the research and clinical communities.

In addition, with the new sample data, we hope to have enough total samples to test our second original research question: Will the concentrations of metabolites in EPS allow us to distinguish between low and high-grade prostate cancer? High grade is defined here as a Gleason score of 7 or above. The ability to distinguish between high and low grade (aggressive vs. less aggressive) prostate cancers would aid clinicians and patients in making treatment decisions. For example, if low-grade cancers could be reliably identified, less aggressive treatments like watchful waiting might be more appropriate for these patients.

Future directions:
Possible future directions for this research include investigating whether citrate, myo-inositol and spermine can be as effectively measured in urine collected after a digital rectal exam rather than EPS collected using an extended prostatic massage. Further, it is hoped that if the current results hold during prospective validation, the metabolic signatures identified by this study may also be less invasively measured using external ^{1}H-NMRS coils, increasing the practicality of the metabonomic approach.

In conclusion, the absolute concentrations of the metabolites citrate, myo-inositol and spermine in EPS are potential age-independent markers of prostate cancer. Age-independent markers of prostate cancer would have great potential in improving the accuracy of screening and early detection. A prospective validation, currently underway, is necessary to confirm these promising results.

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APPENDICES:

Nuclear magnetic resonance spectroscopy of expressed prostatic secretions: Metabolite citrate and derivatives are potential markers of prostate cancer.

Natalie Serkova*, Eduard J Gamito, Richard H Jones, Colin O'Donnell, Tammy Hedlund, E. David Crawford, Denver, CO

Introduction and Objective: Nuclear magnetic resonance spectroscopy (NMRS) along with a novel method for determining absolute concentrations of metabolites were utilized to analyze expressed prostatic secretions (EPS) from men with prostate cancer (PCa) and from healthy controls.

Methods: Flash frozen EPS samples from 66 men (40 with PCa and 26 controls) were analyzed by high-resolution 1H-NMR spectroscopy using a Bruker 500 MHz DRX NMR spectrometer with a 1-mm microprobe. The total number of scans per fully relaxed 1H-NMR spectrum was n=40 with water suppression. Absolute concentrations of endogenous metabolites (citrate, spermine, myo-inositol, lactate, alanine, phosphocholine, glutamate, acetate, hydroxybutyrate) were quantified using trimethylsilyl-propionic acid as an external standard reference. Stepwise multivariable logistic regression (LR) was used to model the risk of PCa based upon the levels of the measured metabolites.

Results: The average age of the EPS donors was 54.7 ± 9.8 years. The median Gleason score for the men with PCa was 6 (range 5-9). The Wilcoxon rank sum test indicated that citrate, spermine, inositol, citrate/spermine, and citrate/lactate were all significant predictors of PCa (p<.001). The LR models indicated that the absolute concentration of citrate was highly predictive of PCa with lower concentrations resulting in a higher risk of cancer. The area under the receiver operating characteristic curve (AUROC) for citrate alone was 0.79 (95% CI 0.75-0.83). Figure 1A shows a PCa probability curve for Citrate. Using relative concentrations (metabolite ratios) in a two-variable LR model, citrate/spermine and citrate/lactate were also predictive of PCa with an AUROC of 0.76 (95% CI 0.71-0.81). Figure 1B shows a plot of these variables and probability of PCa.

Conclusions: The results suggest that absolute concentration of citrate and its derivatives in EPS as measured by NMRS have promising potential as accurate markers of prostate cancer.

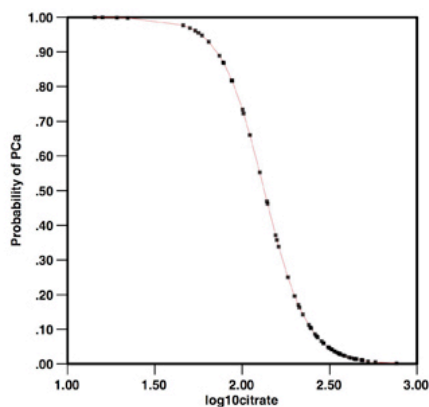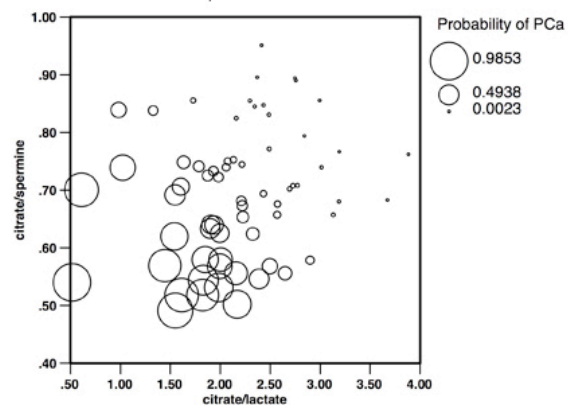Figure 1A: PCa Probability curve for the log10 of citrate.

Figure 1B: PCa Probability based upon citrate/lactate and citrate/spermine ratios.

Validation of citrate and derivatives in expressed prostatic secretions to predict prostate cancer: High-resolution ^{1}H-NMR study

Natalie J. Serkova, *Eduard J Gamito*, *Richard H Jones*, *Colin O'Donnell*, *Tammy Hedlund, E. David Crawford, Denver, CO*

Reliable markers for early detection of prostatic cancer which sensitivity/ specificity will be higher than those of PSA (prostatic specific antigen) are desirable. High concentrations of citrate have been reported in vivo in the healthy prostate gland. Quantitative proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) along with a novel statistical method were utilized to analyze and validate endogenous metabolites in expressed prostatic secretions (EPS) from men with prostate cancer (PCa) and from healthy controls.

Flash frozen EPS samples from 66 men (40 with PCa and 26 controls) were analyzed by high-resolution 1H-NMR spectroscopy using a Bruker 500 MHz DRX NMR spectrometer with a 1-mm microprobe. The total number of scans per fully relaxed 1H-NMR spectrum was n=40 with water suppression. Absolute concentrations of endogenous metabolites (citrate, spermine, myo-inositol, lactate, alanine, phosphocholine, glutamine, acetate, hydroxybutyrate) were quantified using trimethylsilyl-propionic acid as an external standard reference. Stepwise multivariable logistic regression (LR) was used to model the risk of PCa based upon the levels of the measured metabolites.

The average age of the EPS donors was 54.7 ± 9.8 years. The median Gleason score for the men with PCa was 6 (range 5-9). The Wilcoxon rank sum test indicated that citrate, spermine, inositol, citrate/spermine, and citrate/lactate were all significant predictors of PCa (p<.001). The LR models indicated that the absolute concentration of citrate was highly predictive of PCa with lower concentrations resulting in a higher risk of cancer. The area under the receiver operating characteristic curve (AUROC) for citrate alone was 0.79 (95% CI 0.75-0.83). Figure 1A shows a PCa probability curve for Citrate. Using relative concentrations (metabolite ratios) in a two-variable LR model, citrate/spermine and citrate/lactate were also predictive of PCa with an AUROC of 0.76 (95% CI 0.71-0.81). Figure 1B shows a plot of these variables and probability of PCa.

The results suggest that absolute concentration of citrate and its derivatives in EPS as measured by NMRS have promising potential as accurate markers of prostate cancer.

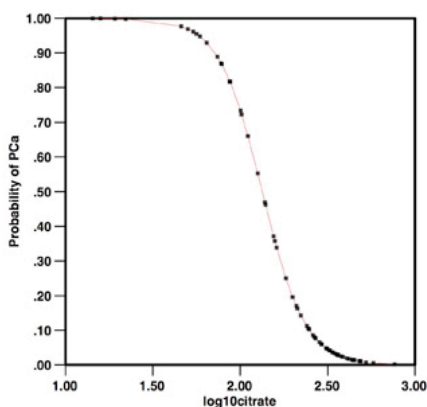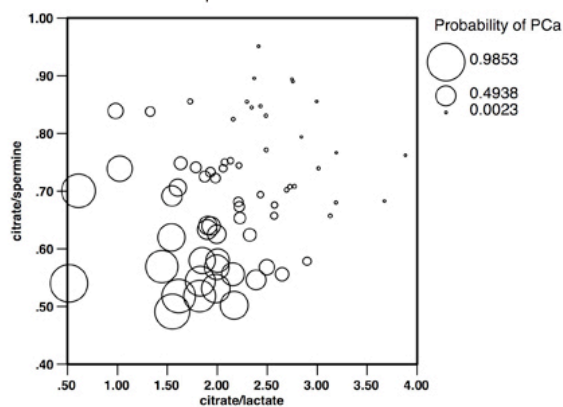Figure 1A: PCa Probability curve for the log10 of citrate.

Figure 1B: PCa Probability based upon citrate/lactate and citrate/spermine ratios.

High-resolution nuclear magnetic resonance spectroscopy of expressed human prostatic secretions: The metabolites citrate, myo-inositol, and spermine are potential age-independent markers of prostate cancer.

Eduard J Gamito (presenter), Natalie Serkova, Richard H Jones, Colin O'Donnell, Tammy Hedlund, E. David Crawford.

Purpose: Due to specific physiological functions, prostatic tissues and fluids have unique metabolic profiles. In this study, proton nuclear magnetic resonance spectroscopy (1H-NMRS) is used to analyze potential metabolic markers of prostate cancer (PCa) in human expressed prostatic secretions (EPS).

Methods: Metabolic profiles of EPS from 52 men with PCa and from 26 healthy controls were analyzed using quantitative MRS. The metabolites analyzed included citrate, spermine, myo-inositol, lactate, alanine, phosphocholine, glutamine, acetate, and hydroxybutyrate. Absolute concentrations were quantified using an external standard reference. Logistic regression (LR) was used to model the risk of PCa based on metabolite concentrations while adjusting for age.

Results: The average age of the EPS donors with PCa was 58.0 ± 7.0 years and 52.2 ± 12.1 for the healthy donors. The median Gleason score for the men with PCa was 6.5 (range 5-9). The LR models indicated that the absolute concentrations of citrate (normal range: 125.9-764.5 µmol/ml), myo-inositol (7.7–41.85 µmol/ml), and spermine (18.9–168.2 µmol/ml) were highly predictive of PCa and inversely related to the risk of PCa. The areas under the receiver operating characteristic curves (AUROC) for citrate, myo-inositol and spermine were 0.89, 0.87, and 0.79, respectively. At 90% sensitivity, these metabolites had specificities of 74%, 51% and 34%, respectively. The LR analysis indicated that absolute levels of these three metabolites were independent of age.

Conclusions: The results indicate that citrate, myo-inositol and spermine are potentially important markers of PCa. Further, the absolute concentration of these metabolites in EPS appears to be independent of age, increasing the potential utility of these markers due to elimination of age as a confounding variable.

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT

1. CONTRACT ID CODE	PAGE OF PAGES	
S	1	2

2. AMENDMENT/MODIFICATION NO.	3. EFFECTIVE DATE	4. REQUISITION/PURCHASE REQ. NO.	5. PROJECT NO.(If applicable)
P00001	02-Mar-2006	W23RYX-4206-N768	

6. ISSUED BY	CODE	W81XWH	7. ADMINISTERED BY (If other than item 6)	CODE	W81XWH
USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014			USA MED RESEARCH ACQ ACTIVITY ATTN: SHANNYN SCASSERO 301-619-2640 SHANNYN.SCASSERO@AMEDD.ARMY.I FORT DETRICK MD 21702		

8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code)		9A. AMENDMENT OF SOLICITATION NO.
UNIVERSITY OF COLORADO HEATLH SCIENCES C FITZSIMONS BLDG 500 MAIL STOP F428 PO BOX 6508 AURORA CO 80045-0508		
		9B. DATED (SEE ITEM 11)
	X	10A. MOD. OF CONTRACT/ORDER NO. W81XWH-04-1-0858
CODE 0P6C1	FACILITY CODE	X	10B. DATED (SEE ITEM 13) 07-Sep-2004

11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS

☐ The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer ☐ is extended, ☐ is not extended.

Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods:
(a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted;
or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE
RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN
REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter,
provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.

12. ACCOUNTING AND APPROPRIATION DATA (If required)

13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS.
IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.

	A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.
	B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).
	C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:
X	D. OTHER (Specify type of modification and authority) USAMRAA General Terms and Conditions for an Assistance Agreement

E. IMPORTANT: Contractor ☒ is not, ☐ is required to sign this document and return _____ copies to the issuing office.

14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.)

Modification Control Number: sscasser061269

1) The purpose of this modification is to extend the period of performance from 15 SEP 04 through 14 APR 06 (Research ends 14 MAR 06) to 15 SEP 04 through 14 OCT 06 (Research ends 14 SEP 06). This action is in accordance with a request from the Awardee dated 1 MAR 06 and the USAMRAA General Terms and Conditons for Assistance Awards. The Grant Officer's Representative has been notified of this modification.

2) No additional funds are required for this extension.

3) All other terms and conditions remain unchanged.

Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.

15A. NAME AND TITLE OF SIGNER (Type or print)	16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print)
	JOSEPH S. LITTLE / CONTRACTING OFFICER
	TEL: 301-619-2546 EMAIL: joseph.little@det.amedd.army.mil

15B. CONTRACTOR/OFFEROR	15C. DATE SIGNED	16B. UNITED STATES OF AMERICA	16C. DATE SIGNED
_____ (Signature of person authorized to sign)		BY *Joseph S. Little* (Signature of Contracting Officer)	01-Mar-2006

EXCEPTION TO SF 30
APPROVED BY OIRM 11-84

30-105-04

STANDARD FORM 30 (Rev. 10-83)
Prescribed by GSA
FAR (48 CFR) 53.243

SECTION SF 30 BLOCK 14 CONTINUATION PAGE

SUMMARY OF CHANGES

SECTION 00010 - SOLICITATION CONTRACT FORM

CLIN 0001

The CLIN extended description has changed from Period of performance: 15 September 2004 - 14 April 2006 (research ends 14 March 2006) to Period of performance: 15 September 2004 - 14 OCT 06 (research ends 14 SEP 06).

 (End of Summary of Changes)